THE ACCOMMODATION OF SOME MARINE INVERTEBRATES TO REDUCED OSMOTIC PRESSURES

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It has been demonstrated by Pearse (1927), Schlieper (1935), Beadle (1931), Sayles (1935) and others that marine forms will survive dilutions of sea water greater than those encountered in their natural environment for various lengths of time. That this ability will be of importance in determining the degree to which a marine species will colonize areas subject to fluctuations in salinity seems likely, but the precise degree of correlation has been little studied. The work reported here consists of two parts: (1) a survey of the distribution of some marine invertebrates in the Narraguagus River estuary, and (2) an experimental investigation on the tolerance to reduced osmotic pressures of the same forms in the laboratory. The entire study was necessarily confined within the period from February, 1940 through April, 1941. The Narraguagus River estuary is located in the town of Millbridge on the coast of Maine. The ecological study consisted of the location of marine species along the estuary, and a study of the freezing point of the water, water temperature, and type of bottom at selected stations.

The experimental work consisted of (1) the determination of the ability of 14 species to live in diluted sea water, (2) a comparative study of the resistance to diluted sea water of specimens of the same species collected from brackish water and from the open sea, and (3) the influence of diluted sea water upon the weight and oxygen consumption of *Nereis virens.* Finally an attempt has been made to correlate the results of the three investigations in an effort to construct conclusions of ecological importance.

Methods and Observations

The Narraguagus River is relatively large near its mouth and is about 300 feet wide at the town of Millbridge. The distance from the open sea to the head of the tide water is approximately ten miles. The

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widening bay into which the river empties is hemmed in by numerous islands and mainland points, and at low tide vast areas of exposed mud flats, ledges, and rocky shores furnish an ideal environment for many common marine invertebrates. With such a body of fresh water entering the sea, it seemed reasonable to assume that there would be regular changes in the salt concentration with the tides, and that sessile forms of life would be subjected to rapid changes in the osmotic pressure of the external medium in their natural environment.

Freezing points were measured in a simple cryoscope by means of a Heidenhain thermometer. Samples were collected at 15 stations (see

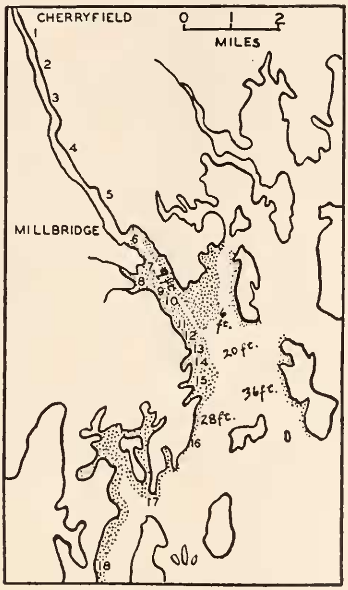

FIG. 1. Map of the lower portion of the Narraguagus River and of Narraguagus Bay, showing points of collections. Station one is at head tide. Stippled areas represent mud flats exposed at low tide. Depths are at mean low tide. Average tide, 11 feet. After U. S. G. S. Cherryfield and Petit Manan quadrangles.

Fig. 1) in small air-tight jars having a capacity of ten cubic centimeters, and were tested in the laboratory after filtering within 48 hours. As can be seen in Table I, the freezing point values at high tide are lower than those at low tide at all points except station one. In general there is a greater depression of the freezing point towards the open sea, although some irregularities appear because of the contours of the channel.

TABLE I

Results of preliminary investigation of Narraguagus River estuary and location of marine species in this region

Station	Freezing point °C.			Temperature		Marine species
	High tide	Low tide	Water extracted from mud	High tide	Low tide	
1	−.011	−.011	....	21	21	
2	−.021	−.014	....	19	20.5	
3	−.141	−.116	....	18.2	20	*Balanus balanoides*
4	−.948	−.145	....	17.5	19.6	*Grammarus* sp. *Crago septemspinosus* *Nereis virens*
5	−1.006	−.404	....	17.2	19.2	*Mya arenaria* *Mytilus edulis* *Electra pilosa* *Flustrella hispida* *Lepidonotus squamatus* *Spirorbis spirorbis* *Acmaea testudinalis* *Littorina litorea* *Thais lapillus* *Modiolus modiolus*
6	−1.014	−.537	−1.345	....	....	*Balanus balanus* *Balanus eburneus* *Jaera marina*
7	−.978	−.438	−1.702	....	....	
8	....	−1.120	−1.386	....	....	*Sertularia* *Hippothoa hyalina* *Cerebratulus lacteus* *Tegella unicornis*
9	....	−1.212	−1.828	....	....	*Glycera dibranchiata*
10	−1.624	−1.236	−1.622	....	....	*Orchestis platensis* *Nucula proxima*
11	....	−1.615	−1.638	....	....	
12	−1.756	−1.374	....	....	....	*Lineus ruber* *Harmothoe imbricata* *Nephthys caeca* *Clymenella torquata*
13	−1.791	−1.491	....	....	....	*Asterias vulgaris*
14	....	−1.706	−1.717	....	....	*Strongylocentrotus drobachiensis*
15	....	−1.771	....	....	....	
16	−1.791	−1.791	....	....	....	*Metridium dianthus* *Tealia crassicornis*
18	−1.791	−1.791	....	....	....	*Bunodes stella* *Clava leptostyla*

The osmotic pressure of the water confined within the mud flats was investigated, since the animals living in the mud are in contact with this confined water rather than the free surface water. A special type of apparatus (Fig. 2) was designed to extract mud at depths optimal for marine worms without surface contamination. A small portion of each end of the core was discarded and the remainder placed in air-tight jars for transportation to the laboratory. Water was extracted by centrifugation and its freezing point was determined. The tubes were covered during this process to prevent evaporation.

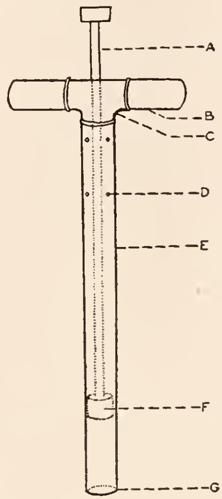

FIG. 2. Mud sampler. *A*. Plunger rod; *B*. Wood handle; *C*. Brass T pipe; *D*. Air expulsion holes; *E*. 1″ brass pipe; *F*. Rubber plunger; *G*. Beveled cutting edge.

The freezing points of this "confined" water from all stations show only slight variations, and differ from those of the surface water in the fact that they do not change much in relation to the distance of the station from the open sea. The variations found may be attributed to differences in the physical qualities of the mud at the various stations, and to the proximity of the station to the river channel. It is notable that in every case the osmotic pressure of the confined water is greater than that of the surface water at high tide. This difference is greatest towards the head of the tide water. It is evident that animals burrowing into mud will not be exposed to the rapid fluctuations in osmotic pressure to which animals attached to the surface are exposed.

At each station the marine species present were collected and identified. In Table I each species is listed under the station farthest up the estuary at which it occurred. It is to be understood that all species were also found at stations nearer the open sea and in greater numbers in these situations. In this work the open sea was considered to be that region at station 16 and beyond where no tidal variation in the freezing point of the water was observed. Particularly noticeable in the low salinity area was the absence of animals belonging to the phyla Porifera, Coelenterata (with the exception of Sertularia), and Echinodermata. However representatives of these phyla were extremely abundant beyond station 15. Another outstanding fact was the size of the animals at station four which were much smaller than similar forms in regions of higher salinity. The worm *Nereis virens* was only two to two and one-half inches long at station four, while it averaged eight inches in the bay proper.

Experiments on Acclimatization to Low Salinity

The experimental animals were brought in from the field and placed in glass dishes of 350 cc. capacity. The dishes were placed in a cooling bath maintained at about 15° C. by means of circulating water. Instead of changing the water in the dishes frequently as other workers have done, air was bubbled continuously through porous "air stones." The entire bath was covered by a glass plate to keep evaporation of the solutions at a minimum. Clean sea water having a freezing point of — 1.791° C. was collected from the open sea at station 16. Dilutions were made with fresh water taken from the Narraguagus River above the dam at station one. After ice formed in the river it was supplanted by Orono tap water. The experiments were started in June, 1940, at Millbridge, and were transferred to the University of Maine laboratories in September. Three series of experiments were carried out.

Opportunity for Acclimatization and Survival of Nereis virens in Fresh Water

Twelve worms of the same size and weight were paired and placed in six dishes containing sea water. Worms were carried down to fresh river water as follows: *A,* from sea water directly into fresh water; *B,* from sea water to 50 per cent sea water to fresh water; *C,* through dilution intervals of 25 per cent; *D,* through dilution intervals of 10 per cent; *E,* through dilution intervals of 5 per cent and *F,* through dilution intervals of 1 per cent. All dilution intervals are stated on the basis of the original concentration, and were made either by adding a calculated amount of fresh water, or by changing the entire medium when the capacity of the dish was reached. A period of 24 hours was allowed for accommodation to each new dilution in groups *A* to *E*. In group *F* the accommodation period was shortened to eight hours, since this period proved long enough to permit adjustment to small alterations in concentration. Control animals were kept in sea water during the entire experiment. The results are given in Table II.

TABLE II

Effects of varying rate of dilution of the external medium on the survival time in fresh water of Nereis virens

Group	Survived 24 hours in each of the following concentrations of sea water	Average survival time in fresh water: hours
A	100 (to fresh water)	6
B	100, 50 (to fresh water)	19
C	100, 75, 50, 25 (to fresh water)	22
D	100, 90, 80 . . . 20, 10 (to fresh water)	32
E	100, 95, 90 . . . 10, 5 (to fresh water)	61
	Survived 8 hours in the following concentrations of sea water	Average survival time in 2% sea water
F	100, 99, 98 . . . 3.2*	72

* Limit of survival in this experiment.

It will be noted that *Nereis* was able to survive in fresh water for longer periods of time as the acclimatization process was made more gradual. Although the animals in this experiment were not weighed, the increased body size and turgidity of the worms due to water intake was very evident in the cases where the dilution interval was large. This turgid condition was less noticeable as the dilution interval became smaller. In group *E* it was not observed until the worms had passed below the 25 per cent sea water level. Subsequently group *F* reached 10 per cent sea water before this condition became apparent. This would indicate that *Nereis* can accommodate to reduced osmotic pressure if the change is gradual enough. It should be noted that the worms in group *F* died in 2 per cent sea water after surviving in that concentration for 72 hours. Work was necessarily suspended at that time so that the survival time in fresh water could not actually be ascertained. At very low salinities frequent changes of the water were necessary, even with a liberal amount of air being supplied. This was due to what seemed to be an excessive expulsion of coelomic fluid which caused putrefaction of the medium. There is a possibility that this fluid was eliminated through the nephridia. The control animals were in good condition when the experiment was discontinued 42 days after it was started.

The Comparative Susceptibility of Fourteen Species to Reduced Osmotic Pressure

Fourteen species representing the phyla Coelenterata, Nemertea, Annelida, Arthropoda, Mollusca and Echinodermata were selected because of their availability in the bay. The apparatus and methods were the same as those described above. Dilutions were made through 1 per cent intervals every eight hours until the animals appeared to have reached their critical point for survival, as indicated by sluggishness and turgidity. The time between changes was then increased to 72 hours. The number of animals in each dish varied between one and 25 depending upon the size. In most cases only two or three were confined in the same dish.

Table III records the results of this investigation. The freezing point values listed are those of the water at low tide at the station where the animals were collected. These areas were selected for collecting specimens because of the numbers available. All animals were in excellent condition when brought to the laboratory with the exception of *Tealia crassicornis* which could not be removed from the rocks without injury to the pedal disc. It should be stated too that it was very difficult to keep *Asterias vulgaris* for any length of time even in natural sea water. The control animals lived very little longer than the experimentals. It is probable that their brief survival was due to other factors. One such factor was nutrition. Even though whole clams and clear chopped clam tissues were placed with them, they failed to eat.

There was considerable variation among individuals of the same species in their abilities to survive in low concentrations of sea water. In a few instances the difference between the minimum and maximum lethal concentrations is high (e.g. *Mya arenaria*), but in most cases the difference is 10 per cent or less. In general it was found that the smaller animals of each species were better able to survive than the larger members. Four *Nereis virens* lived in 1 per cent sea water 52 hours, and three *Gammarus* lived in fresh water for 72 hours. *Glycera dibranchiata* became very swollen and turgid in 50 per cent sea water, and in greater dilutions the integument in certain body regions burst causing extensive loss of blood. *Jaera marina* survived in fresh water for two weeks, and were discarded to make room for other experiments.

On the basis of these results a general phyletic relationship can be established in regard to the ability of animals to survive in reduced salt concentrations. Such a classification stated in order from greatest to least ability of survival is: Arthropoda, Annelida, Mollusca, Nemertea, Echinodermata and Coelenterata. With the exception of Coelenterata, this order conforms roughly to the degree to which these phyla have succeeded in colonizing fresh water.

Table III

The comparative abilities of fourteen species to withstand reduced osmotic pressures arranged in order of decreased resistance

Animal	Freezing point of water in collecting areas	Number used	Per cent sea water withstood −1.791° C.		
			Min.[1]	Max.[2]	Average
Jaera marina	−1.791	25	..	..	0
Gammarus sp.	−1.212	18	0.5	10	2
Nereis virens	−1.120	12	2	2	2
Crago septemspinosus	−0.438	4	3	5	4
Balanus balanoides	−1.212	4	7	13	9
Mytilus edulis	−1.212	8	2	25	9.6
Thais lapillus	−1.212	6	6	24	13
Balanus balanus	−1.212	3	10	18	14
Glycera dibranchiata	−1.212	8	30	38	35
Mya arenaria	−1.120	6	8	55	36
Cerebratulus lacteus	−1.120	6	42	58	47
Asterias vulgaris	−1.791	6	66	84	73
Clava leptostyla	−1.791	2 col.	71	76	74
Tealia crassicornis	−1.791	6	80	90	85

[1] Minimum percentage concentration below which no animals survived for 72 hours.

[2] Maximum percentage concentration above which no animals died within a 72-hour period.

A Comparison of Individuals from the Open Sea and from Brackish Water in Their Resistance to Diluted Sea Water

In this experiment members of four species, collected from areas exposed to the open sea (station 18), were carried through a procedure as described in the preceding section. Their survival was compared with that of brackish water forms collected from station seven. The results of this experiment are shown in the upper part of Table IV with results extracted from Table III added so that an effective comparison can be made. It will be seen readily that in every case the open sea individuals were less resistant to changes in the concentration of sea water than the brackish water forms. The difference is less pronounced in some species, and suggests a higher development of the osmotic regulatory mechanism in these species. The comparison may be made still more significant by the fact that after open sea specimens of *Mya* had succumbed, brackish water clams introduced into the same concentration of sea water survived and were able to adjust down to a limit of 10 to 8 per cent sea water.

TABLE IV

The comparative abilities of similar open sea and brackish water forms to withstand reduced osmotic pressures

Animals from open sea areas Δ = −1.791	Number used	Dilution per cent sea water		
		Min.	Max.	Average
Nereis virens..........	6	6	8	7
Gammarus sp..........	9	3	16	8
Mytilus edulis..........	5	39	46	43
Mya arenaria..........	8	48	58	52
Animals from brackish water Δ = −.978				
Nereis virens..........	12	2	2	2
Gammarus sp..........	18	0.5	10	2
Mytilus edulis..........	8	2	25	9.6
Mya arenaria..........	6	8	55	36

Effects of Gradually Reduced Osmotic Pressure on the Weight and Oxygen Consumption of Nereis virens

According to Beadle (1931) *Nereis diversicolor* increases in weight on transfer to dilute sea water and also increases its respiratory rate. Sayles (1935) likewise found an increase in the weight of *Nereis virens* in dilute sea water, but did not determine the respiratory rate. In their experiments the worms were transferred directly to water of low salinity. In the experiment here described five animals were transferred daily through 4 per cent dilution intervals. The Rideal-Stewart modification of the Winkler method was used to determine the amount of dissolved oxygen in the water in closed vessels before the worms were placed in them, and after they were removed. The worms were kept in the closed containers four hours, during which period the apparatus was darkened so as to keep activity at a minimum. Figure 3 shows the results of this experiment. The average weight of the worms shows a slight but consistent decrease even down to 50 per cent sea water. Then there is a marked increase in weight at 28 per cent sea water, but a change to still more gradual dilutions brought the weights down again.

The final weight rise beginning at 16 per cent appeared to be due to the breakdown of the osmoregulatory mechanisms, and was characterized by the intake of large amounts of water which made the animals turgid. Up to this point the animals remained firm with no sign of excess water either in the coelom or the integument.

The results of the oxygen consumption measurements show much more variation between individual worms. This is probably due to differences in the amount of activity. A rather definite trend of the averages is evident, however, and this is correlated with the weight

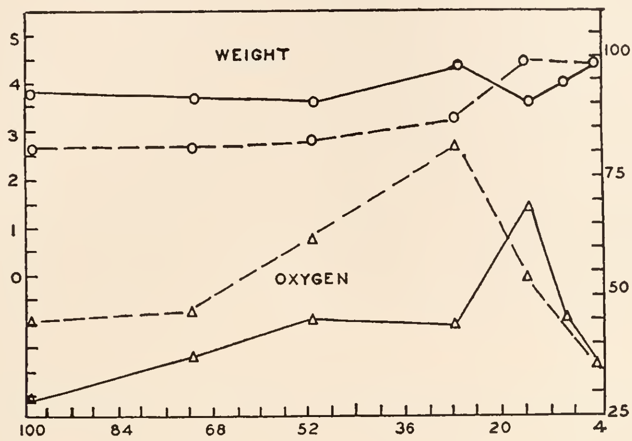

FIG. 3. Effects of diluted sea water on the weight and oxygen consumption of *Nereis virens*. Left hand ordinates represent total weight of five worms in grams; right hand ordinates represent average cu. mm. oxygen per gram per hour. Abscissa; percentage concentration of sea water. Continuous line represents readings as animals were carried from 100 per cent sea water to 4 per cent. Dashed line represents the reverse experiment.

curve. With moderate dilutions the weight remained constant, but oxygen consumption rose 33 per cent. In the experiment described below a corresponding but much sharper decrease in oxygen consumption was found as the worms were returned to normal sea water. We believe that the increased oxygen consumption in dilute sea water represents the expenditure of energy for osmotic work. Below the critical range of 28 to 16 per cent sea water there was a decrease in the amount of oxygen used per gram per hour. Since weight was increasing during this period it is difficult to separate the decrease due to the intake of metabolically inactive water from that which may be expended in osmoregulation.

Weight and Oxygen Consumption of Acclimatized Nereis virens when Gradually Returned to Natural Sea Water

The same animals used in the experiment described above were carried from 4 per cent sea water back to natural sea water, but necessarily at a more rapid rate. Tests of oxygen consumption were made at the same concentrations as in the previous experiment except at 10 per cent sea water. Apparently osmotic regulation is restored as the osmotic pressure of the medium is increased. This is indicated by the sudden decrease in weight accompanied by a marked increase in oxygen consumption up to the critical point at 28 per cent sea water. From that concentration up to full strength sea water there is a direct relationship between the change in weight and the amount of oxygen consumed for, as there is less need for regulation, less energy is expended by the animals. Particularly noticeable at the end of this experiment was the fact that the average weight of the worms had declined 38.5 per cent from the original value. Some of this loss may be due to loss of salts, but since the animals were used experimentally over a period of 94 days, it is probable that other features of an artificial environment may have contributed to this condition.

Discussion

Experimentally all brackish water animals except *Balanus balanoides* were able to survive in media of lower osmotic pressure than those encountered in their natural environment. It may be that the eggs or juvenile stages are less resistant to dilution of the sea water, so that the species fails to invade the less saline areas. It is also possible that the animals avoid water below a certain salt concentration even though they can live in it. In the Narraguagus a complication is found in the presence of accumulations of sawdust from mills located on the river. This is abundant as far south as station four, and undoubtedly has an unfavorable effect on many marine organisms.

According to Adolph (1925) most marine animals are able to live after abrupt change to almost pure fresh water provided that the remaining salts are present in physiological proportions, and gradual dilutions over several days do not materially help the animals to endure pure water. Although he states that some species lived in 1 per cent sea water he names no specific organisms. This is in disagreement with the results of other workers. Pearse (1927) was able to carry few species into solutions weaker than one-fourth sea water, while most of the 18 forms he worked with could not survive in concentrations less than 50 per cent sea water. Sayles (1935) found the minimum survival concentration for *Nereis virens* to be between 30 and 40 per cent sea water. In these experiments it has been possible to obtain better results than some other workers by keeping the animals at a relatively constant temperature approximating that of their natural environment, maintaining constant aeration, and preparing dilutions with fresh water collected from the Narraguagus River. Dilutions made with distilled water were found to be definitely toxic, while Orono tap water did not give as good results as river water.

Other work bearing on the effect of salinity changes upon respiration appears to be scarce. Beadle (1931) reports that Turussov (1927) found by carbon dioxide measurements with a modified Osterhout apparatus that the respiratory rate of *Nereis diversicolor* increased in hypotonic and decreased in hypertonic sea water. Beadle himself and Schlieper (1935) confirmed these results, but Krogh (1939) states that the small increase in oxygen consumption shown in their calculations does not exceed the limits of error, and therefore cannot be regarded as significant. He admits that energy must be expended for osmotic work, but claims that the amount is too small for demonstration. In view of the unknown efficiency of this process, however, the prediction may not be valid.

Both Beadle and Sayles report that the weights of several *Nereis diversicolor* and *Nereis virens* paralleled one another for a time, but that some would soon decrease in weight at the same time that others were increasing. In many cases those animals that continued to increase in weight became turgid with a resulting rupture of the body wall allowing considerable loss of body fluid. The weights of *Nereis virens* in the experiments here reported showed very little variation, and then only in the extremely low salinities, while no animals were observed to become swollen to the bursting point. *Glycera dibranchiata,* on the other hand, took on large amounts of water in concentrations of 50 per cent sea water and in 39 per cent sea water the integument burst with considerable loss of blood and internal fluids.

A comparison of the relative ability of the three species of *Nereis* which have been tested is difficult because of differences in technique. *Nereis virens* appears to use less oxygen per gram weight than do the other two species, but on the basis of surface area the amounts consumed are about the same.

Summary

1. The distribution of 14 species of invertebrates in the Narraguagus Bay and River was compared with the osmotic pressure (indirectly de-

termined by means of freezing points) of the water at a series of stations ranging from the open sea to the head of the tide.

2. The osmotic pressure of the water confined in the mud flats was found to be almost constant at high and low tides in contrast with the marked tidal variations in surface water.

3. Typical marine animals were found in an environment having a freezing point of — .404° C., or the equivalent of 19 per cent sea water.

4. The survival of *Nereis virens* in fresh water was extended as the process of acclimatization was made more and more gradual.

5. Tests on 14 species showed that all but one were able to survive experimental exposure to reduced osmotic pressure below that found in their natural environment.

6. Members of a species collected from water of low salinity were more resistant to reduced osmotic pressure than individuals collected from the open sea.

7. *Nereis virens* was able to accommodate without marked change of weight down to a concentration of 16 per cent sea water. At this point a rapid increase in weight occurred. The dilution process above 16 per cent was accompanied by an increased oxygen consumption. Below 16 per cent sea water oxygen consumption decreased.

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